

Appl. No. : 09/802,397
Filed : March 9, 2001

C3
1'20'') 40 cycles, 72°C 10' and for P1A: 94°C 2' (94°C 30'', 55°C 30'', 72°C 30'') 35 cycles, 72°C 10' were in a Perkin-Elmer/Cetus DNA thermal cycler. Primers used were as follows: actin sense primer 5'-TGCTATCCAGGCTGTGCTAT-3' (SEQ ID NO: 5), actin antisense primer 5'-GATGGAGTTGAAGGTAGTTT-3' (SEQ ID NO: 6), P1A sense primer 5'-GGGACCATGGCCCACAGTGGCTCAGGT-3' (SEQ ID NO: 7) and P1A antisense primer: 5'GGGGGATCCTTAGACAGAGGACATGCGCTTG-3' (SEQ ID NO: 8), resulting in an amplified fragment of 240 bp.

REMARKS

Amendments were made to identify nucleotide sequences recited in the specification according to the numerical identifiers indicated in the Sequence Listing. The amendments above were previously requested in the amendment filed by the Applicants on September 16, 2002. No new matter is added herewith. Changes made to application can be seen on a separate page entitled VERSION WITH MARKINGS TO SHOW CHANGES MADE following the signature page. Insertions are underlined.

Should any issues arise which may delay prosecution of the present application the Examiner is respectfully invited to contact the undersigned. Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated:

Oct. 25, 2002

By:

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Insertions are underlined.

IN THE SPECIFICATION:

Please amend the paragraph beginning on page 40, line 18, as follows:

Next, the Polymerase Chain Reaction (PCR) was used to detect rearranged V b8-Cb sequences of the TCR in genomic DNA. The upstream primer was targeted to bases 47-66 with respect to the ATG initiation codon of the mouse V b8 region (5'-AACACATGGAGGCTGCAGTC-3' (SEQ ID NO:1)) and the downstream primer was targeted to bases 141-160 of the first exon of the Cb region (5'-GTGGACCT CCTTGCCATTCA-3' (SEQ ID NO:2)). The PCR was carried out essentially according to the instructions of Boehringer Mannheim's Long Range Expand PCR System. Analysis of the PCR products on a 1% agarose gel stained with ethidium bromide is shown in Figure 3. A fragment with the expected length (4.5 to 5 kb) of the rearranged Vb8-Cb fragment is clearly seen in DNA from the T-cell hybridoma 13-26-8-H6 (lane T), used as a positive control, as well as in DNA from the HY41 and HY62 hybridomas (lanes 41 and 62); this fragment is not amplified in DNA from P815* tumor cells and from spleen cells (lanes P and S), used as negative controls. These results confirm that the DLC that fused with a P815* tumor cell to yield the HY41 and HY62 hybridomas was a T-lymphocyte expressing an a/b TCR receptor, including the V8 domain. These hybridomas will hereafter be termed T-DLC/tumor cell hybridomas.

Please amend the paragraph beginning on page 44, line 15, as follows:

The goal of these experiments was to determine whether the HY41 and HY62 hybridomas synthesized some cytokines that could account, at least in part, for their in vivo immunogenicity. Total RNA was prepared from activated spleen cells, from P815* tumor cells and from the HY41 and HY62 hybridomas according to standard procedures. The Reverse-Transcription Polymerase Chain Reaction (RT-PCR) and cytokine-specific primers were used to amplify IL-2, IL-4, IL-10 and interferon γ (IFN- γ) mRNA sequences, as described by De Wit et al, J. Immunology, 1993, 150: 361-366. The primers used to amplify IL-12 p40 sequences were 5'-TTCAACATCAAGAGCAG TAGC-3' (SEQ ID NO: 3) and 5'-GGAGAAGTAGGAATGGGGAGT-3' (SEQ ID NO: 4). Analysis of the RT-PCR products on ethidium bromide-stained agarose gels showed that P815* tumor cells constitutively expressed

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IL-4 mRNA and that the HY41 and HY62 hybridomas constitutively expressed IL-2 and IL-4 mRNAs, but not IL-10, IL-12, and IFN γ mRNAs. These cytokine mRNAs were nevertheless detected in activated spleen cells, used as a positive control. In conclusion, these data showed that the HY41 and HY62 T-DLC/tumor cell hybridomas constitutively expressed IL-4 like the parent P815* tumor cell, and IL-2, like the parent T-lymphocyte. These cytokines, if secreted in vivo, may at least partially contribute to the immunogenicity of the hybridomas.

Please amend the paragraph beginning on page 55, line 21, as follows:

Total RNA was extracted from P815 and hybrid cells using TRIZOL reagent (total RNA isolation reagent, Gibco BRL, Merelbeke, Belgium). Less than 1 μ g RNA was used to perform a control PCR for actin and a P1A gene specific PCR with the TitanTM One tube RT-PCR System (Boehringer Mannheim, Brussels, Belgium). The cDNA synthesis was performed following the manufacturer's instructions. The PCR reactions for actin: 94°C 2' (94°C 30'', 60°C 30'', 72°C 1'20'') 40 cycles, 72°C 10' and for P1A: 94°C 2' (94°C 30'', 55°C 30'', 72°C 30'') 35 cycles, 72°C 10' were in a Perkin-Elmer/Cetus DNA thermal cycler. Primers used were as follows: actin sense primer 5'-TGCTATCCAGGCTGTGCTAT-3' (SEQ ID NO: 5), actin antisense primer 5'-GATGGAGTTGAAGGTAGTTT-3' (SEQ ID NO: 6), P1A sense primer 5'-GGGACCATGGCCCACAGTGGCTCAGGT-3' (SEQ ID NO: 7) and P1A antisense primer: 5'GGGGGATCCTTAGACAGAGGACATGCGCTTG-3' (SEQ ID NO: 8), resulting in an amplified fragment of 240 bp.